R. WHITMORE, H. JONES, W. WINDUS and J. NAGHSKI
USDA Eastern Marketing & Nutrition Research Div., ARS
Eastern Regional Research Lab., Philadelphia, PA 19118

PREPARATION AND VISCO-ELASTIC PROPERTIES OF FIBROUS COLLAGEN DISPERSIONS FROM LIMED CATTLEHIDE SPLITS

INTRODUCTION

COLLAGEN is a high molecular weight, insoluble fibrous protein. As a nonwoven fabric in the form of hides and skins it is ideal for the manufacture of leather. However, the less desirable parts must be disposed of in other ways. Trimmings are processed into gelatin, glue and tankage. In the production of shoe-upper leather the hide is split to produce the desired thickness from the grain side. The split from the flesh side is usually in surplus, although at the moment it is being made into cheap, coarse suede for garments for the younger set. Hides themselves may become a surplus commodity. At present the United States is successfully exporting 15 million cattlehides a year and this number is increasing.

Fibrous collagen has some unique physical and chemical properties. However, the natural state within the hide does limit its utility. Numerous workers have attempted to modify the hide structure physically and chemically in an effort to convert the fiber to useful products (Braun and Braun, 1956; Cohen, 1964; Cresswell, 1953; Halloran, 1935; Reissmann and Nichols, 1960; Runkel and Lange, 1937; Schulte, 1936). Such work has resulted in the successful dispersion of hide collagen fibers and regeneration of tubular casings suitable for sausage making (Fagan, 1968; Kidney, 1970; Lieberman, 1968; Talty, 1969a, b). Several of the early workers did not distinguish between limed and unlimed collagen, others described processing of unlimed, raw, fresh hides while others (Fagan, 1968; Kidney, 1970; Lieberman, 1968; Talty, 1969b) specifically included lime-unhaired hides as raw material. In early work Runkel and Lange (1937) specified hide that had been limed for a long time (2–4 wk) as raw material. In the work reported here, the raw material was obtained from commercially-limed hide processed under conditions that pulped the hair in a short time and was split to remove the grain layer (upper 50%). It was again split to remove the adhering fat and flesh to produce a very clean corium split.

Lime treatment causes changes in collagen which are readily recognizable, but poorly defined (Gustavson, 1956). This sets limed collagen apart from fresh or salt-cured collagen from hide or other sources. Also, the short liming treatment leaves the collagen in less separated structure with more of its amide nitrogen, glycoproteins and mucopolysaccharides than the long-limed collagens (Eastoe, 1967). Thus the purity of the hide collagen used in these experiments is limited by its history (Deasy, 1959). Therefore, one preparation of collagen was used in experiments reported here.

Dispersions of acid-swelled collagen are often described in the patent literature (Schulte, 1936; Runkel and Lange, 1937; and later patents). Viscometric behavior of soluble collagen has been reported by Cerny et al. (1970) and Kahn and Witnauer (1966). It is important to exclude acid soluble filtrates and neutral salt solutions from consideration in this paper, since limed hide has little, if any, of either component. We have not found reference to warm neutral dispersions of previously limed fibrous collagen in the literature. Recent work has not included study of alkaline dispersions either cold or warm. This paper compares some visco-elastic properties of dispersions of fibrous collagen from previously limed hide corium prepared under cold and warm conditions at three levels of pH.

EXPERIMENTAL

Raw materials

Fresh splits from commercially limed and unhaired cattlehides were used as raw material. The splits were washed, delimed, neutralized and again washed to a pH of approximately 6.5. Drained of superficial water, the splits were successively cut in (a) rotary blade cutters and (b) an Urschel Comitrol fitted with a nominal .060 in. cutting head as described previously (Elias et al., 1970; Whitmore et al., 1970). The comminuted particles have a maximum dimension of 0.04–0.08 in. The product obtained from eight splits contained 28.7% collagen; the fat content was 10% on a dry basis. Fat was determined by acetone extraction, in a Soxhlet apparatus. Solids were based on the residue after heating for 16 hr at 105°C. Ash was 0.67% on dry solids. The lot was divided into 100 and 1000g aliquots frozen separately in impervious, sealed food bags for use in these experiments.

Adjustment of pH

Aliquots of the comminuted collagen were suspended in water; the pH was adjusted to a constant value by adding solutions of 10% lactic acid or 10% sodium hydroxide while stirring either by hand or at a very low speed in a Variac-controlled Waring Blendor. Water was added to make the suspension 6% collagen w/v.

Dispersion

Dispersions were made by means of high speed mixing in a Waring Blendor or in a valve homogenizer. The Waring Blendor was used at high speed for preparing small batches of dispersions but air was often trapped in the form of small bubbles. These bubbles could be removed from dilute dispersions only.

To avoid the presence of air bubbles, the Manton-Gaulin valve homogenizer was used to treat larger lots of 6% collagen. During operation of the homogenizer, 80 lb air pressure was applied to the feed tank, while the two homogenizing valves were set to apply about 1500 psi at each stage. Dilutions of the 6% collagen dispersions were made in the Blendor at low speed to avoid air entrapment.

Dispersions made while holding temperatures below 30°C were designated as "cold" dispersions; those made while holding temperatures above 40°C were designated as "warm" dispersions. Both types of dispersions were prepared at three pH levels: acid (pH 3.7); neutral (pH 7.0); and alkaline (pH 12.6).

Viscosity

"Viscosities" were measured with a Brookfield HBT Viscometer using a helipath stand when necessary. "Viscosity" thus measured cannot be construed as true viscosity. True viscosity is usually measured in a homogeneous medium such as motor oil or a warm gelatin solution. Our dispersions are not homogeneous in this sense; what is measured by the Brookfield Viscometer is more or less the resistance of the particles in the dispersion to the action of a stirring bar or disc. (The bar often became clothed with fiber, thus the readings are somewhat suspect of moderate error.)

Gel strength

The gel strengths of the various types of dispersions were measured after chilling at 8°C for 16 hr by means of a Marine Colloids Inc. Gel Tester with 0.25-in. diam ram.

RESULTS

COMMINUTED HIDE when suspended in aqueous media yields preparations that show a wide variation in consistency depending on the pH and temperature of the suspending medium and the mechanical action applied during preparation. Collagen suspended in an aqueous medium of low ionic strength and a pH below 4.1 swells to as much as 100 times its dry volume (Elden, 1958; Veis, 1964;

Cassel and McKenna, 1954) and can be easily dispersed. Above pH 4.3, collagen does not swell appreciably until well into the alkaline pH range. No sharp break occurs in the swelling curve however, and we have arbitrarily chosen the value pH 8.5 as the cut-off for the "neutral" range. Except for the effect of heat, dispersions made throughout this range were similar.

Acid dispersions with lactic acid as swelling agent had a pH of 3.7. Alkaline dispersions required a pH of about 12.6 when sodium hydroxide was used, to insure adequate swelling for cold dispersion without additives. Satisfactory cold dispersions could not always be made in the pH range from 4.3 to about 8.5 without using an additive such as guar gum. Fibrils appeared to become intertwined and reaggregated in this pH range until the temperature had risen to a transition point (observed to occur at between 50–70°C depending on the nature of the suspending medium).

This gave rise to a study of "warm" dispersions. It was found that a rise in temperature alone was not responsible for the transition, but that high shear was also necessary. Suspensions heated to 50°C or higher in a water bath, without stirring and in the pH range 4.3–8.5 were easily separable. Dispersions, formed when high shear was applied by the Waring Blendor or the homogenizer, were shown to contain fibers by diluting and centrifuging. Sedimented material was fibrous under dark field illumination in the microscope at about 100 diameters magnification. The supernatant was assumed to be gelatin, but addition of a gelatin solution to a suspension at 40°C did not aid dispersion. When mechanical energy of the blendor had raised the temperature to the usual transition point, the dispersions with or without added gelatin passed through a coarse sintered glass filter while warm. Warm dispersions were made at pH 3.7 and pH 12.6 for comparison.

It was felt that "viscosity" measurements would reflect the differences in consistency. Table 1 gives the results comparing preparations containing 6% collagen and dispersed under cold and warm conditions at three pH levels. As would be expected, the cold dispersions are more viscous than the corresponding warm preparations. It was also noted that the acid-swelled fibers have increased viscosity over neutral dispersions of the same concentration, while the warm alkaline dispersions are more fluid than corresponding neutral dispersions.

Mechanical action has a pronounced effect on the consistency of collagen preparations. Results of "viscosity" measurements made on suspensions containing 6% collagen before and after passing through a Manton-Gaulin valve homogenizer are shown in Table 2.

"Viscosities" increase generally with high shear treatment, except with warm alkaline preparations. The suspended particles before treatment affect the readings on the Brookfield, since the suspensions are not homogeneous. The liquor in which the particles are suspended shows little change in viscosity. Brookfield viscometer dial readings are converted to centipoises by using a given factor with each spindle and speed. With the wide ranges of "viscosity" encountered in a series of dilutions, it was frequently found necessary to change both spindle and rotational speed.

The effect of collagen content of dispersions on the consistency of preparations was investigated at pH 3.7 and 7.0. Dilutions were made by adding water at the appropriate pH to the dispersions containing 6% collagen and mixing briefly in a Waring Blendor at low speed. The procedure was adopted to minimize differences induced by any additional shear during the dilution operation.

Viscosity measurements on the dispersions are plotted in Figure 1. Cold dispersions at 6% solids, pH 3.7 are much less affected by dilution than are warm dispersions of the same pH.

Cold acid dispersions were difficult to characterize under this system. In instances where more than one spindle and speed could be applied, changes in speed gave a wide range in centipoise values as shown in Figure 1 by the dashed portion of the vertical bars where the measurements were made. At the 0.25% concentration, only the largest spindle available gave readings in the useful portion of the scale. Therefore only the data obtained by changing speeds are presented. At one speed, changing the spindle gave smaller variation in values at each concentration. The results obtained with different spindles are shown as the solid portion of the vertical bars. The curve shown is a "best fit" between these scattered values to allow comparisons to be made with the more consistent values shown for warm acid and warm neutral dispersions. Time after stirring had little effect on readings from cold acid preparations. Temperature changes of 10C degrees between 10°C and 30°C had little effect on readings from the cold acid dispersion. Also, variations of 10C degrees between 40°C and 60°C had little effect on "viscosities" of warm acid and neutral dispersions.

Collagen dispersions appeared to show thixotropy as evidenced by an increase in the apparent viscosity with time. This was most apparent with dispersions made under warm conditions. The response of a typical dispersion studied at 50°C and at a pH of 7.0 is shown in Figure 2. Thixotropy, measured at about 50°C on the Brookfield Viscometer, is shown in this curve. It was not reduced by warming. In earlier experiments we were not aware of the magnitude of this thixotropic effect and its bearing upon comparisons between dispersions. Over the 80-min observation period the viscosity had increased about fivefold and was still rising. "Viscosity" readings for this reason were made as quickly as possible after stirring had stopped. Time after mixing was considered a more critical variable than the temperature of the dispersion. Most dispersions of hide collagen show considerable gelling power when chilled. The gel strength of dispersions containing 6% collagen and made at 6 different conditions are presented in Table 3. The products of cold dispersion at all pH values tested show low gel strength compared to those of warm dispersions in the neutral range and at pH 3.7. Warm, strongly alkaline dispersions do not form gels on cooling. Gels from warm dispersions at pH's ranging from 3.7–8.5 show

Table 1—Brookfield "viscosities" of 6% collagen dispersions made under various conditions

pH	"Cold" 25°C	"Warm ' 40°C
3.7	3,200,000 cps	80,000 cps
7.0	Viscosity not consistent; Guar gum added	800 cps
12.6	1,200,000 cps	< 64 cps

Table 2—"Viscosities" of acid and alkaline dispersions, before and after homogenizing under cold and warm conditions

	"Cold"	"Warm"
At pH 3.7		
Before homogenizing	270,000 cps	35,000 cps
After homogenizing	3,200,000 cps	80,000 cps
At pH 12.6		
Before homogenizing	128,000 cps	6,400 cps
After homogenizing	1,200,000 cps	< 64. cps

Table 3—Effect of pH and conditions of preparation on the gel strength of dispersions containing 6% collagen[a]

pH	"Cold" (g)	"Warm" (g)
3.7	120	800
7.0	75	1000
12.6	22	0

[a]Measurements were made on a Marine Colloids Inc. Gel Tester after cooling for 16 hr at 8°C. A sample with a Gelatin Bloom Rating of 262 read 450 on this instrument.

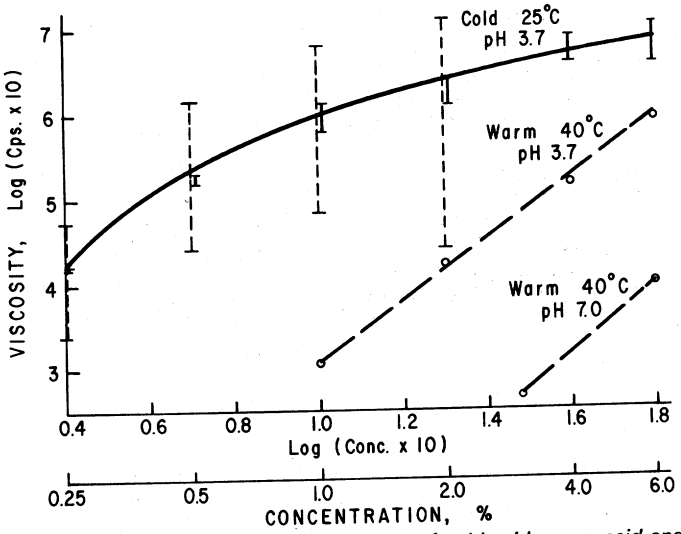

Fig. 1—A comparison of the "viscosities" of cold acid, warm acid and warm neutral collagen dispersions at 6% and lower collagen concentrations. Solid I bars show the variation in Brookfield viscosity values [log (cps × 10)] obtained on cold acid dispersions using different spindles at 5 rpm; the dashed portions of the I bars show the variation obtained with the single spindle that gave readings over the range in speed from 0.5—100 rpm.

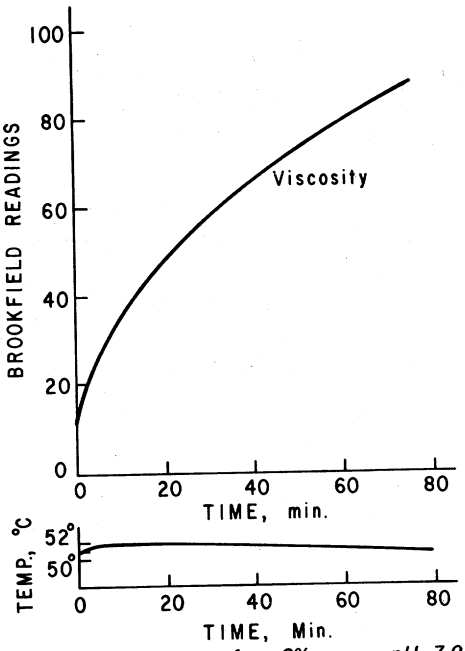

Fig. 2—Thixotropy of a 6% warm pH 7.0 dispersion. Readings shown were taken from a Brookfield viscometer using an HBI spindle at 5 rpm.

gel strength about twice that of gelatin of 62 Bloom.

DISCUSSION

IT IS APPARENT that dispersions of collagen can be made in a variety of ways to produce characteristics ranging from a fibrous paste to a liquid virtually free of fibers. Control of temperature and concentration adds further to the versatility of the products. Warm dispersions have been made at > 30% collagen solids, but cold acid and "neutral" dispersions could not always be produced above 6% collagen concentration. Accordingly, comparisons could not be made.

Fibrils of native collagen undergo changes in the various dispersions determined by pH, temperature and ionic strength. At low ionic strength (salt concentration below 1%) cold acid dispersions consist almost entirely of swollen fibrils (Borysko, 1963) with no free fluid apparent at concentrations as low as 0.5%. When the pH is raised, a sudden change occurs at about pH 4.3. The fibrils seem to collapse to the unswollen state, freeing water and reaggregating into a ropy, fibrous mass. This is especially noticeable with stirring. This phenomenon occurs with all dispersions in the 4.3–8.5 pH range. Reaggregation is reduced by introducing additives, or by heating to approximately 40°C or above, but even then, the stability of the dispersions is not assured.

Native fibrils can be found in the high shear dispersions up to temperatures of 50°C to 70°C in the 4.3–8.5 pH range, but most fibrils have shrunk at 65°C to less than 25% of their original length, and lose the 700A spacing of native collagen (Nutting and Borasky, 1948).

Alkaline dispersions tend to lose fibrous structure at elevated temperatures. At 47°C a sodium hydroxide dispersion at pH 12.6 lost fibrous structure to give the lowest viscosity reading of the six samples tested. This dispersion did not gel when cooled.

Cold dispersions retain the shrink behavior of fibrous collagen. Cold acid dispersions have thixotropic behavior that results in a soft gel at concentrations of 0.5% or above, but it does not appear as quickly or as strongly as it does in warm dispersions. Consecutive readings on the Brookfield fall noticeably while the spindle is turning but usually recover on standing.

The thixotropy of warm neutral dispersions shown in Figure 2 may affect viscosity readings on dilutions (Fig. 1) in that the onset of increased viscosity with time may be quicker in concentrated dispersions than in dilute dispersions. We are planning dynamic studies of viscosity in higher solids dispersions which may shed some light on this effect if it is present. The thixotropic property of warm neutral dispersions is advantageous for extrusion forming. In addition, the strength of these gels when cooled is much greater than that of gelatin. They may serve as lubricants and binders when added to flours and meals used for extrusion mixtures or pellets, in amounts as little as 2%. The formed product swells in water but does not dissolve or disintegrate. Catfish and shrimp feed is an example of a promising application of these properties.

The warm neutral dispersions after cooling to a firm gel at 6% solids did not dissolve in hot water. The gels became tender, did not shrink, and to our surprise, did not dissolve in boiling water. It appears, in view of these properties and the thixotropic behavior, that thermal relaxation (Banga, 1966) or renaturation (Verzar, 1965) may occur or that the fibrous component begins to crosslink following high shear treatment. A higher solids gel retains its form during boiling.

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Ms received 7/1/71; revised 12/28/71; accepted 12/29/71.